REMARKS

Claims 4, 14, 21, 22, 47-49, 64, 65, 73-82, and 127-142 were previously canceled. Claims 1, 5, 16-18, 20, 23, 25-19, 33-36, 50-63, 66-72, 82-126, 143, and 152-164 have been withdrawn as being directed towards non-elected subject matter. Applicants reserve the right to file divisional or continuing applications directed towards the canceled subject matter. Claim 11 has been amended. Support for the amendment can be found throughout the specification, specifically in the claims as originally filed. No new matter has been added. Claims 1-3, 5-13, 15-20, 23-46, 50-63, 66-72, 83-126, and 143-166 are currently under consideration.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 2, 3, 6-13, 15, 19, 30-32, 144-151, 165, and 166 are rejected under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. Specifically, the Examiner states the while the specification is enabling for:

> (1) orally administering to said first mouse colitis extracted proteins (CEP) prepared from colons that were removed from TNBS-induced-colitis mice, cut into small strips, mechanically homogenized, filtered through a 40 mm nylon cell strainer, and the colitis extract supernatant isolated from intact cells via centrifugation;
>
> (2) obtaining 0.5×10^6 liver associated lymphocytes and 2.5×10^6 splenocytes from a second mouse that had been treated with TNBS to induce colitis and had been orally administered CEP prepared as in step (1);
>
> (3) adding to a culture of the 0.5×10^6 liver associated lymphocytes and 2.5×10^6 splenocytes from step (2) antigen presenting cells and CEP as in step (1);
>
> (4) optionally adding to said culture IL4, IL-10, TGFβ, IL 18 or IL 15;
>
> (5) administering the cultured cells of step (3) to the first mouse in need of such treatment to modulate the Th1/Th2 balance towards anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ration between any one of IL4 and IL

10 to IFNγ

does not reasonably provide enablement for

a method for the treatment of immune-related or immune-mediated disorders or disease in a mammalian subject in need of such treatment, by manipulation of the NKT cell population of said subject, wherein manipulation of said NKT cell population results in modulation of the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, said modulation being mediated by different components, cells, tissues or organs of said subject's or another subject's immune system comprising the steps of:

a. obtaining NKT cells from said subject or another subject;
b. *ex vivo* education of the NKT cells obtained in step (a) such that the resulting educated NKT cells may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells; and
c. re-introducing to said subject the educated NKT cells obtained in step (b) which may modulate the Th1/Th2 cell balance toward anti-inflammatory cytokine producing cells, resulting in an increase in the quantitative ration between any on of IL4 and IL 10 to IFNγ,

wherein said *ex vivo* education of step (b) is performed by culturing said NKT cells in the presence of any one of:

a. antigens or epitopes associated with said immune-related or immune-mediated disorder or disease to be treated, antigens or epitopes associated with the immune-mediated inflammatory response, or any combination thereof;
b. at least one liver-associated cell of tolerized or non-tolerized subjects suffering from said immune-related or immune-mediated disorder of said subject;
c. at least one-cytokine or adhesion molecule, or any combination thereof; and
d. a combination of any of step (a), (b) and (c)

and wherein said NKT cells may optionally express the CD56 marker.

Office Action pages 5-6. The Examiner cites to several references to contend that there is uncertainty in the state of the art concerning the subject matter of the instant specification. The Examiner further contends that the specification broadly reads on a method comprising treatment wherein the only cell present in an NKT cell (Office Action page 10) and that it is unclear if *ex vivo* education in the presence of CEP would exert any effect on NKT cells

(Office Action page 10). The Examiner then concludes that "The specification does not enable the genus because where the results are unpredictable; the disclosure of a single species usually does not provide an adequate basis to support generic claims." Office Action page 16.

35 U.S.C. §112, first paragraph requires that a specification enable one skilled in the art to make and use the claimed invention. A specification fails to meet this requirement if the specification fails to provide sufficient information regarding the claimed subject matter to enable a skilled artisan to make and use the claimed invention. "However, to comply with 35 U.S.C. §112, first paragraph, it is not necessary to 'enable one of ordinary skill in the art to make and use a perfected, commercially viable embodiment absent a claim limitation to that effect.' *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338, 68 USPQ2d 1940, 1944 (Fed. Cir. 2003)." (MPEP §2164). To determine if sufficient information is provided, one must inquire whether the claimed invention can be practiced without undue experimentation. MPEP §2164.01. That some experimentation may be required is not fatal because the issue is whether the experimentation is undue. *In re Vaeck*, 20 USPQ2d 1438, 1444 (Fed. Cir. 1991).

Applicants respectfully traverse the rejection and assert that the claims are enabled for the full scope of the claims. The present specification contains only one example directly concerned with *ex vivo* training to shift immune responses from Th1 to Th2. In contrast, initial examples described in WO 02/051986 (hereinafter "the '986 application") are concerned with the induction of oral tolerance and the ability to transfer such tolerance into another subject by transferring NKT cells from a donor subject that has undergone tolerance induction by exposure to an appropriate antigen. In this situation, it is viewed that shift in the Th1/Th2 balance to an anti-inflammatory response in the donor is passed along to the recipient after such a transfer. The Examiner appears to agree that oral administration of CEP alleviated symptoms, a process which involved NK1.1 cells. Office Action page 9.

The fact that these transfers provided high success rates in the resulting shifts in immune responses leads to the conclusion that although induction of the shift took place after exposure of the antigens to membranes in the gut, a direct exposure of the antigens to NKT cells *in vitro* should create a population of NKT cells that would induce a similar effect. As such, transfer of NKT cells that have been "educated" *in vitro* by antigen exposure should induce the same effects as NKT cells that have been "educated" *in vivo* by antigen exposure

(oral tolerance induction). As will be discussed below, this hypothesis was tested in Example 7 and the results demonstrate accordance of effects between NKT cells that have undergone *in vivo* training by oral tolerization and NKT cells that have undergone *ex vivo* training.

The Examiner states the following on page 9 of the Office Action: "Given all of the data from the instant specification seems to be identically disclosed in prior art reference WO 02051986 of Yaron Ilan..." While the present specification contains all of the examples that are described in the '986 application, it is supplemented with a large amount of new data derived from other examples that expand the teachings of the '986 application. It appears that the Examiner does not appreciate the presence of this additional information, particularly with regard to its relevance to the pending claims. This relevancy will be discussed in more detail below.

The Examiner concludes from the data of E"2, E"3, E"5, and E"6 that there is no obvious consistent effect. Office Action page 10. Applicants respectfully disagree with the Examiner's characterization of the experimental data. In evaluating this Experiment, it should be understood that E"2, E"3, E"5, and E"6 are the only members of Group E that have been treated with TNBS and therefore represent a diseased state. E"2 is a control where there is no treatment. Group E"3 is another control group where a previously described treatment, induction of oral tolerance is used. This is a control since it represents a previous method that has been shown to alleviate inflammation by the present inventors as well as by other groups (as cited in the specification). A comparison of E"2 and E"3 indicates that oral tolerization of the E"3 subjects does provide benefits when there is a very large increase in the amount of IL10 compared to the no treatment control (E"2). The E"5 group indicates that substitution of *ex vivo* education of the NKT cells (the new method) can present a palliative effect that is similar to oral tolerance (the old method). In other words, the methodology used in the E"5 group offers a large increase in IL10 secretion with only a small amount of IFNγ induction. In fact, this conclusion is specifically spelled out in the discussion of this experiment:

> As shown by Table 6, culturing NK1.1+ T cells in the presence of disease associated antigens (subgroup E"5) leads to **cytokine patterns that is similar to that of tolerized cells** as manifested by increased IL10 secretion". (emphasis added)

Here, the E"3 group is used as a standard for achieving success in inducing an anti-inflammatory state. Additionally, this experiment demonstrates that this state may also be achieved by the *ex vivo* education (as shown by the E"5 group). The exact value of the IFNg/IL10 is not important so much as the directionality, i.e., is IFNγ>IL10 or is IL10>IFNγ? Thus, a large increase in IL10 as illustrated in the E"5 group is achieved while maintaining a high ratio of IL10>> IFNγ. The E"6 group is a separate experiment that shows that the use of both treatments (oral tolerance and *ex vivo* education) is in fact counterproductive.

Although the above-described experiment was present in the '796 disclosure, its significance should be understood in the context of the new Examples that are present in the current specification. As mentioned above, the rationale for performing the *ex vivo* training was to investigate whether such a methodology could replicate effects of *in vivo* training by oral tolerance induction. Clearly, the results in Table 6 indicate that NKT cells which have been trained by either method are effective in ameliorating inflammatory markers and producing a shift in the Th1/Th2 balance. Consequently, one of skill in the art would recognize that when subsequent experiments are performed with *in vivo* training, these results would also be obtained by *ex vivo* training as well.

As mentioned above, the experiments illustrated in the present specification supplement the experiments described in the '986 disclosure (the '986 application contains seven examples, the present specification continues with more data). For instance, the present specification provides an example utilizing a ConA model of liver inflammation and the oral administration of liver extracts to reduce inflammation, i.e., shifting the Th1/Th2 balance. See specification, page 25, Part III (US Patent Application Publication No. 20050069546). Due to the previous experiments with CEP extracts, the success with *in vivo* education would be predictor of likely success with *ex vivo* education.

The Examiner states that "'an NKT cell' is not just one type of cell but a genus of cells with differing cell surface markers, differing antigen-presenting molecule restriction specificities and in turn differing modes of activation and differing effector functions." Office Action page 11. Applicants respectfully assert that term "NKT cells" represents a variety of different subtypes. The present invention is not limited to particular subtypes. The NKT population as a whole was used in Example 7 for use in *ex vivo* education and in numerous other Examples where the properties of NKT cells on metabolic and

immunological effects were observed. The present invention des not require knowledge of which particular subgroups of NKT cells may be responsible for these effects.

The Examiner further states that it is too unpredictable to determine which antigens associated with an immune-related or immune-mediated disorder would be capable of inducing NKT dells to modulate the Th1/Th2 cell balance. Office Action page 11. The present invention does not require one either identify the particular component(s) or isolate the particular component(s) in the CEP that induced both in vivo and *ex vivo* training of the NKT cells. All that is required for the production of the appropriate effects is the presence of the antigen a sufficient amount in the material that is administered.

The Examiner cites several references in order to categorize the state and predictability of the art. For Example, Margalit *et al.* is cited to as describing "disappointing results". Margalit was published as a response to a comment by Das 2006 (Am J Gastroenterol 101; 2889-2890) concerning a previous article Margalit *et al.* (Am J Gastroenterol 2006 101 561-568). However, the original Margalit *et al.* article describes some moderate levels of success in terms of the rate of clinical remission (58 % vs. 29%) and improved quality of life (43% vs. 12%). It is apparent that the method did work but the success rate was not as high as would be desired. As discussed in another comment on this article (Hyun and Barrett. 2006 Am J Gastroenterol 101; 569-571), the original Margalit report represents preliminary results and "Larger scale studies using variable dosages, modes, and durations of Ag delivery will be required to optimize oral tolerance therapy in IBD.) (emphasis added)

The Examiner again references a "particular NKT cell" and indicates the presence of a requirement of specific subgroups of NKT cells. Office Action pages 12-14. Applicants again respectfully assert that this not required in the presently claimed invention. The Examiner further cites Doherty where CD56 cells are described as Th1 producing cells. Office Action page 14. Applicants assert that Doherty is not applicable to Applicants presently claimed invention which recites a shift in the Th1/Th2 balance. As such, one of skill in the art would understand that prior to "education" the NKT cells may possess aTh1>Th2 cell balance.

The Examiner cites to Kaneko *et al.* as evidence that IL-4 appears to augment cytotoxic effects in the ConA model. Office Action page 14. Applicants note that this paper is concerned with the influence of IL4 on induction of inflammation and remarks that effects

from IL4 result through "an autocrine fashion". As such, there is no indication that, after induction, a rise in IL4 level derived from treatment of mice with *in vivo* or *ex vivo* educated NKT cells would necessarily augment symptoms which took place during induction. As noted by the Examiner, the Vα14 cells used in Kaneko *et al.*, are in fact a particular subset. Thus, treatment of the entire population of NKT cells may have a different effect than treatment of the one particular subset used by Kaneko.

The Examiner states that using cytokins such as IFNγ or IL12 in the claims would be antithetical to the claim method. Office Action page 14, citing to Leite-De-Moraes. Applicants respectfully contend that the claims are fully enabled as written. However, solely in an attempt to further prosecution, claim 11 has been amended to delete the reference to the objected cytokines.

The Examiner states that the addition of adhesion molecules such as integrins, selectins or ICAMs would be unpredictable. The Examiner continues in stating:

> The expression of the integrin LFA-1 on a liver associated cell is known to be essential for NKT cell homing to the murine liver so adding LFA-1, which could compete with LFA-1 in a liver associated cell for binding to the ex vivo "educated NKT cells could prevent the administration of NKT cells from getting to where they may need to be in order to induce bystander expression of auto reactive CD4+ T cells.
>
> Likewise, the skilled artisan would be hard pressed to predict the effect that the addition of ICAN, such as ICAM-1 may have on claimed method because based upon the teachings of Emoto the skilled artisan would not know with any degree of certainty if addition of ICAM-1 would for example block the interaction of NKT expressed LFA-1 with liver Kuppfer cells

Office Action page 15, citing to Emoto, Abstract and page 5097, last paragraph. Emoto reads "In the *livers* of LFA-1-deficient mice, the number of $CD4^+$NKT cells was markedly decreased. This reduction was restricted to the liver, and no reduction was found in the other organs analyzed." See Abstract. Thus, the findings of Emoto are not applicable to the Applicants' invention as the presently amended claims are not restricted to liver cells.

The Examiner continues in stating:

> Likewise, as is well know to the skilled artisan, E-selectin is involved in homing of lymphocytes to the skin, and given that some human Vα24i NKT cells express an E-selectin ligand, it is unclear how ex vivo educated NKT cells could be used, for

> example, to treat skin diseases after being cultured in the presence of a selectin which could compete with the skin expressed E-selectin for the homing of a subset of ex vivo educated human NKT to the skin where they may need to be in order to induce bystander suppression of auto-reactive CD4+ cells.

Office Action page 15, citing Kim *et al.* The treatments recited in Kim are restricted to skin cells while the presently amended claims are not restricted to this cell type. Neither the Emoto nor Kim references are applicable to the teachings of Applicants' presently claimed invention. These references are restricted to the treatment of two specific organs (liver and skin), while the present claims recite a method for the treatment of an *immune-related* or *immune-mediated* disorder. Therefore, the teachings of Emoto and Kim are not a proper indication of the state of the predictability of the art as applied to the present invention.

Applicants respectfully assert that the claims are enabled as currently amended. Contrary to the Examiner's contentions, the specification provides adequate instruction to allow one of skill in the art to make and use the invention. As acknowledged by the Examiner, the oral administration of CEP did show the alleviation of symptoms. In addition, the specification teaches accordance of effects between NKT cells that have undergone *in vivo* training by oral tolerization and NKT cells that have undergone *ex vivo* training. The Experimental results as described in the specification indicate that NKT cells which have been trained by either method are effective in ameliorating inflammatory markers and producing a shift in the Th1/Th2 balance. In addition, the present invention is not limited to a particular subset of NKT cells, nor does the invention require one to either identify the particular component(s) that induced both in vivo and *ex vivo* training of the NKT cells. That some experimentation may be required to practice an invention is not fatal to patentability. Instead, one must inquire whether the claimed invention can be practiced without *undue* experimentation. Here, Applicants' specification provides adequate guidance to practice the invention without undue experimentation. Withdrawal of the rejection is respectfully requested.

Rejection Under 35 U.S.C. §102(b)

Claims 2, 3, 6-13, 15, 19, 32, 144-151, 165, and 166 are rejected under 35 U.S.C. §102(b) as being anticipated by the '986 application. The Examiner states that the '986 application teaches the claimed invention, in particular a method of treating Crohn's disease.

Office Action page 17.

Applicants respectfully traverse the rejection. For a rejection under 35 U.S.C. §102 to be properly made and sustained, the art cited in that rejection must disclose each and every element of the claim(s) called out in the rejection. MPEP §2131. The presently amended claims are directed to the subject matter not described in the '986 application. The present specification teaches that that the use of both oral tolerance and ex vivo education is in fact counterproductive. As described above, the present specification indicates that NKT cells which have been trained by either method are effective in ameliorating inflammatory markers and producing a shift in the Th1/Th2 balance. The '986 application does not teach each and every limitation of the present claims. Withdrawal of the rejection is respectfully requested.

CONCLUSION

Applicants respectfully submit that all claims are in condition for allowance. Early notification of a favorable consideration is respectfully requested. In the event any issues remain, Applicants would appreciate the courtesy of a telephone call to their counsel at the number listed below to resolve such issues and place all claims in condition for allowance.

Respectfully submitted,

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